nuclear run on, Northern blotting, Western blotting and luciferase reporter assay, respectively. Transcription factor binding activity was assessed by EMSA and chromatin immunoprecipitation (ChIP).
Results: TSA strongly reduced MIF mRNA and protein levels in whole blood and cell lines. Nuclear run on analyses showed that TSA reduced MIF gene transcription. Yet, TSA affected neither the activity of an ectopically expressed MIF promoter, nor the nuclear content of Sp1 and CREB, two transcription factors previously shown to be indispensable for MIF gene expression. Surprisingly, ChIP analyses revealed that, even though global histone acetylation was strongly increased by TSA, TSA deacetylated the histones associated with the MIF promoter. This effect required protein synthesis and was coupled with a decreased recruitment of Sp1 and CREB to the bona fide MIF promoter.
Conclusions: TSA down-regulates MIF expression by a molecular mechanism involving a local deacetylation of MIF-associated histones and a reduced transcription of the MIF gene. Considering that MIF is over-expressed in human tumors and required for tumor associated angiogenesis, our findings suggest that the anti-tumoral effects of HDIs may be mediated by a down-regulation of MIF expression.

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Anti-Toll-Like Receptor 4 (TLR4) Antibodies Protect from Lethal Endotoxemia but not from Gram-negative Septic Shock

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Background: TLR4, an essential signalling component of the LPS receptor complex plays a critical role in innate immune defenses against Gram-negative (GN) bacteria, suggesting that it might be a candidate target for therapeutic interventions in patients with GN septic shock.
Objective: To investigate the effects of neutralizing anti-TLR4 antibodies in murine models of lethal endotoxemia and GN septic shock.
Methods Rabbit anti-mTLR4 IgG, raised against a soluble recombinant chimeric protein composed of the extracellular domain of mouse TLR4 fused to the Fc domain of human IgG1 (mTLR4-Fc), or control IgG were tested for their ability: (1) to bind to mTLR4-Fc and native TLR4 by ELISA or FACS, (2) to inhibit LPS-induced cytokine production in whole blood assay or by primary macrophages, and (3) to protect OF1 mice from lethal endotoxemia (50 ng of LPS i.v. with D-galactosamine) or E. coli O18 sepsis (10^4–10^5 CFU i.p.) with or without ceftazidime (CAZ) (500 mg/kg i.v. 12–16 h after E. coli and then q12h).
Results: Anti-mTLR4 IgG recognized mTLR4-Fc and macrophage TLR4, and inhibited LPS-induced TNF production in vitro. In vivo results are presented in the table.

	Anti-TLR4 IgG		Control IgG	
	TNF (ng/ml)	Alive/total (survival)	TNF (ng/ml)	Alive/total (survival)
LPS shock	0.13 (0–0.65)*	16/17 (94%)+	1.3 (0.05–20)*	7/15 (47%)+
E. coli sepsis (w/o CAZ)	0 (0–0.9)	0/6 (0%)	1.1 (0.0–3.2)	1/6 (17%)
E. coli sepsis (with CAZ)	ND	1/22 (5%)	ND	2/22 (9%)

IgG: 0.5–2.0 mg 30 min before LPS or 2–16 h after E. coli; TNF: 1 h after LPS, 3 h after E. coli; ND: not done; *,+P < 0.005.

Conclusions: Anti-mTLR4 IgG protected mice from lethal endotoxemia, but not from Gram-negative septic shock. Thus, whereas TLR4 is critical for LPS sensing, other signalling pathways are also activated during Gram-negative sepsis, suggesting that immunomodulating strategies just aimed at blocking TLR4 might not suffice to improve the outcome of patients with Gram-negative septic shock.

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Role of Toll Like Receptors in Post Viral Disease

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Background: Toll Like Receptors (TLRs) are pattern recognition proteins in mammalian cells that are responsible for the fever, hypotension, and shock that follow some bacterial and viral infections. By serving as signal transduction proteins, TLRs recognize foreign antigens in viruses and bacteria and stimulate cells to make cytokines. These cytokines are responsible for the acute fever and other early manifestations of infection. In addition, they are critical in the development of the acquired immune response. While the role of TLRs in the initial immune response has been documented for many different infections, less studied has been the role that these proteins have in virus associated "autoimmune" or post viral responses.
Objectives: To investigate the role of TLRs in the response to *Coxsackie B3 virus*.
Methods: Human peripheral blood lymphocytes or dendritic cells from inbred mice missing TLR genes were stimulated with *Coxsackie B3* (CB3) virus with and without antibodies to CB3 and supernatants were assayed for the production of cytokines.